



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>A61K 31/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 00/30629</b> <b>(43) International Publication Date:</b> 2 June 2000 (02.06.00)
<b>(21) International Application Number:</b> PCT/US99/27634 <b>(22) International Filing Date:</b> 19 November 1999 (19.11.99)  <b>(30) Priority Data:</b> 09/199,912                      25 November 1998 (25.11.98)      US  <b>(71) Applicant (for all designated States except US):</b> INSPIRE PHARMACEUTICALS, INC. [US/US]; Suite 470, 4222 Emperor Blvd., Durham, NC 27703 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> PENDERGAST, William [GB/US]; Suite 470, 4222 Emperor Blvd., Durham, NC 27703 (US). SHAVER, Sammy, R. [US/US]; Suite 470, 4222 Emperor Blvd., Durham, NC 27703 (US). DRUTZ, David, J. [US/US]; Suite 470, 4222 Emperor Blvd., Durham, NC 27703 (US). RIDEOUT, Janet, L. [US/US]; Suite 470, 4222 Emperor Blvd., Durham, NC 27703 (US).  <b>(74) Agent:</b> HALLUIN, Albert, P.; Howrey & Simon, Box 34, 1299 Pennsylvania Avenue, N.W., Washington, DC 20004 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished          upon receipt of that report.</i>
<b>(54) Title:</b> METHOD OF PROMOTING CERVICAL AND VAGINAL SECRETIONS  <b>(57) Abstract</b>  The present invention provides a method of stimulating cervical and vaginal secretions in a mammal by treatment with P2Y <sub>2</sub> , and/or P2Y <sub>4</sub> purinergic receptor agonists. Treatment of vaginal dryness associated with menopause, chemotherapy, and various disease states as well as the treatment of vulvar pain is discussed. Suitable purinergic receptor agonists include nucleotide triphosphates such as uridine 5'-triphosphate, cytidine 5'-triphosphate and adenosine 5'-triphosphate; dinucleotide polyphosphates such as P <sup>1</sup> , P <sup>4</sup> -di(uridine-5') tetraphosphate; and their analogs. These purinergic receptor agonists are useful in stimulating cervical and vaginal secretions and treating vaginal dryness in mammals.		

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## METHOD OF PROMOTING CERVICAL AND VAGINAL SECRETIONS

### BACKGROUND OF THE INVENTION

The mucus covering on the surfaces of the female reproductive tract is  
5 important in its defense and reproductive function. The mucus gel, secreted primarily  
by the endocervical epithelium, provides a barrier to sperm and pathogen penetrance  
into the endometrium and a protective covering for the vaginal epithelium. Hydration  
of vaginal and cervical mucus prevents atrophy, provides lubrication during  
intercourse, aids surface defense against pathogens, and modulates sperm entry into  
10 the uterus, etc. ( see Gipson I.K., *et al.*, *Biology of Reproduction*, 60, 58-64 (1999))

Vaginal and ectocervical epithelia, are a squamous epithelia (similar to skin),  
which do not contain ciliated cells. The vaginal epithelium is sloughed off during  
certain phases of the menstrual cycle. The lower endocervical epithelium contains  
submucosal glands consisting of mucin secreting columnar epithelial cells, but it does  
15 not have ciliated cells. The upper endocervix has the same cells as the lower  
endocervix and also contains ciliated cells. The role of the ciliated cells in endocervix  
is not mucociliary clearance. At the time of ejaculation, spermatozoa become  
suspended in the vaginal and cervical secretions, which are then actively mixed by the  
action of cilia beating and contraction of vaginal musculature. This involves to-and-  
20 fro movement of columns of cervical mucus so that spermatozoa can migrate into the  
mucus. One role of ciliated cells in endocervix is to expel nonviable or dead sperms;  
the cilia beat towards the vagina and the viable sperm swim against the gradient.

Vaginal dryness is a very common problem which brings physical and  
emotional distress to many women (Key, E., *Nurs. Stand.* 5:24-27 (1991)). It most  
25 commonly manifests itself during sexual intercourse, which causes dyspareunia and  
can eventually lead to apareunia. Although it is traditionally considered to be a  
condition which affects postmenopausal women, it can occur during the  
premenopausal and perimenopausal years. The use of oral contraceptives may also  
cause a reduction in vaginal moisture in some women (Reginald, W., *et al.*, *Br. J.*  
30 *Obstet. Gynaecol.* 96:1148-1152 (1989)). Postpartum vaginal dryness, independent of  
or as a result of lactation, can be a significant complaint (Wisniewski, P., *et al.*, *Am. J.*  
*Obstet. Gynecol.* 165:1249-1254 (1991)). Women undergoing chemotherapy or  
radiotherapy for malignant diseases such as leukemia often experience vaginal  
dryness as a result of treatment (Cust, M., *et al.*, *Br. Med. J.* 299:1494-1497 (1989)).

Many disease states, such as systemic sclerosis and other systemic autoimmune disorders (Bhadauria, S., *et al.*, *Am. J. Obstet. Gynecol.* 172:580-587 (1995)), Ehlers-Danlos syndrome (Sorokin, Y., *et al.*, *J. Reprod. Med.* 39:281-284 (1994)), diabetes mellitus (Sreebny, L., *et al.*, *Diabetes Care* 15:900-904 (1992)), and Sjögren's syndrome (Marchesoni, D., *et al.*, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 63:49-53 (1995)) have decreased vaginal hydration and lubrication problems as significant disease-associated symptoms.

Vulvar pain is defined as the excessive sensitivity of the nerves supplying the mucus membrane of the vulva. This persistent burning and sensitivity in vulvar skin is not caused by identifiable infection. It cannot be cured by surgery. The diseases covered under "vulvar pain" are also referred to as vulvodynia/vulvar vestibulitis, vulvitis, burning vulvar syndrome and is often associated with fibromyalgia, irritable bowel syndrome, Sjögren's syndrome, chronic inflammation, and Paget's disease as well as in the absence of any identifiable disease or infection. R. Paul St. Armad, M.D., an endocrinologist at UCLA, has successfully treated fibromyalgia with uricosuric (gout) drugs, especially guaifenesin, a drug used to liquefy mucus (Yount, J.J. *et al.*, *Women's Health Digest* 3(2) 1997). Dr. Armad has found that such gout drugs provide an effective treatment for fibromyalgia, even though gout and fibromyalgia have no connection. Dr. Armad has found that 24-hour urine samples taken from patients before and after treatment exhibited a significant increase in the excretion of phosphate and a moderate increase of oxalate and calcium after guaifenesin was started. His hypothesis is that an excess of intracellular phosphate, and possibly oxalate, builds up in the cells of fibromyalgia sufferers and depresses formation of energy (ATP) in the mitochondria of the cells. It should be noted that the role of ATP in Dr. Armad's theory is as an energy source and not an agonist of the P2Y<sub>2</sub> receptor.

Current therapies for increasing vaginal moisture are: lubricating agents such as lubricating creams or jellies, topical estrogen creams, and HRT (hormone replacement therapy). Lubricating jellies provide short-lived and temporary relief, as these are aqueous preparations containing no pharmacologically active agent. The effect of estrogen on maintenance and function of female genitalia has been well-documented. Thickness and rugae of the vaginal wall, as well as vaginal lubrication are both estrogen dependent. Estrogens have also been shown to increase pelvic blood flow in menopausal women and women with surgical or medical ovariectomy.

Estrogen deficiency results in vaginal mucosal atrophy and vaginal dryness, which results in symptoms of dyspareunia, sexual dysfunction, as well as a variety of urogenital complaints. Although estrogen therapy is effective in many women, it may be contraindicated in others due to medical reasons such as breast cancer. For example, Topical estrogen creams, if used on a regular basis, may be absorbed into the systemic circulation. This can cause endometrial stimulation and can lead to endometrial hyperplasia and carcinoma (Whitehead, M., *et al.*, *N. Eng. J. Med.* 305:1599-1605 (1981)). HRT is effective at relieving symptoms of vaginal atrophy and hence vaginal dryness but has several contraindications and unwanted risks and side effects. A history of gall bladder disease (*N. Eng. J. Med.*, 290:15-19 (1974)) or a personal or family history of reproductive or breast cancer (Harlap, S., *Am. J. Obstet. Gynecol.* 166:1986-1992 (1992)) are contraindications for estrogen therapy. Other contraindications are: history of stroke, cardiovascular disease, deep-vein thrombosis, superficial thrombophlebitis, liver disease, heavy smoking, high blood pressure, diabetes, uterine bleeding or large fibroids, hyperlipidemia, and gross obesity (Lichtman, R., *J. Nurse Midwifery* 36:30-48 (1991)). One major disadvantage of HRT is the resumption of monthly withdrawal bleeds, which many postmenopausal women will not accept. Some women, even while on HRT, still experience a degree of vaginal dryness (Key, E., *Nurs. Stand.* 5:24-27 (1991)).

It has been shown that uridine 5'-triphosphate (UTP) and dinucleotide polyphosphates such as diuridine tetraphosphate are potent agonists of P2Y<sub>2</sub> purinergic receptors found on the surface of human airway epithelium. UTP has been shown to increase both the rate and total amount of mucin secreted by goblet cells *in vitro* (Lethem, M., *et al.*, *Am. J. Respir. Cell Mol. Biol.* 9:315-322 (1993)). UTP has also been shown to increase chloride secretion, and hence, water secretion from airway epithelial cells *in vitro* (Mason, S., *et al.*, *Br. J. Pharmacol.* 103:1649-1656 (1991)).

Diuridine tetraphosphate has been shown to have beneficial properties in the treatment of various diseases, such as chronic obstructive pulmonary disease (COPD). For example, they have been demonstrated to facilitate the clearance of mucous secretions from the lungs of a subject such as a mammal including humans in need of treatment for various reasons, including cystic fibrosis, chronic bronchitis, asthma, bronchiectasis, post-operative mucous retention, pneumonia, primary ciliary dyskinesia (M. J. Stutts, III, *et al.*, U.S. Patent No. 5,635,160; PCT International

Publication WO 96/40059) and the prevention and treatment of pneumonia in immobilized patients (K. M. Jacobus and H. J. Leighton, U.S. Patent No. 5,763,447). Further therapeutic uses include treatment of cystic fibrosis, chronic bronchitis, asthma, bronchiectasis, post-operative atelectasis and Kartagener's syndrome (PCT International Publication WO 96/40059), sinusitis (PCT International Publication WO 98/03177), otitis media (PCT International Publication WO 97/29756), dry eye, retinal detachment, nasolacrimal duct obstruction, the treatment of female infertility and irritation due to vaginal dryness via increased mucus secretions and hydration of the epithelial surface, and enhancing the performance of athletes.

As a result of the ineffectiveness and risks of current therapies for vaginal dryness, medical researchers have sought to develop alternative treatments. Because of the demonstrated ability of UTP and dinucleotide polyphosphates, such as diuridine tetraphosphate, to increase hydration of airway epithelial secretions and stimulate release of mucins, applicants were motivated to investigate whether UTP and dinucleotide polyphosphates could stimulate hydration and mucin production in the vaginal and cervical epithelia.

### **SUMMARY OF THE INVENTION**

A method of stimulating cervical and vaginal secretions in a subject in need of such treatment is disclosed. The method of the present invention may be used to increase cervical and vaginal secretions for any reason, including, but not limited to, treatment of vaginal dryness and/or treatment of vulvar pain. Vaginal dryness is associated with but not limited to menopause, childbirth, breastfeeding, chemotherapy or radiotherapy, diabetes mellitus, Sjögren's syndrome, Ehlers-Danlos syndrome, systemic sclerosis and other systemic autoimmune diseases, hysterectomy, urogenital surgery, psychosomatic disorders, anxiety, psychosexual problems, and pharmacological drug-related side effects. The method of the present invention comprises administering a P2Y<sub>2</sub> and/or P2Y<sub>4</sub> purinergic receptor agonist: uridine 5'-triphosphate (UTP), cytidine 5'-triphosphate (CTP), adenosine 5'-triphosphate (ATP), P<sup>1</sup>,P<sup>4</sup>-di(uridine-5')tetraphosphate, or their analogs thereof, in an amount effective to stimulate vaginal and cervical secretions.

Another aspect of the present invention is the use of uridine 5'-triphosphate, cytidine 5'-triphosphate, adenosine 5'-triphosphate, P<sup>1</sup>,P<sup>4</sup>-di(uridine-

5')tetraphosphate, or their analogs thereof, for the manufacture of a medicament for carrying out a therapeutic method of treatment as given above.

The present invention also discloses pharmaceutical compositions comprising uridine 5'-triphosphate, cytidine 5'-triphosphate, adenosine 5'-triphosphate, P<sup>1</sup>,P<sup>4</sup>-di(uridine-5')tetraphosphate, or analogs thereof, with a pharmaceutical carrier therefor.

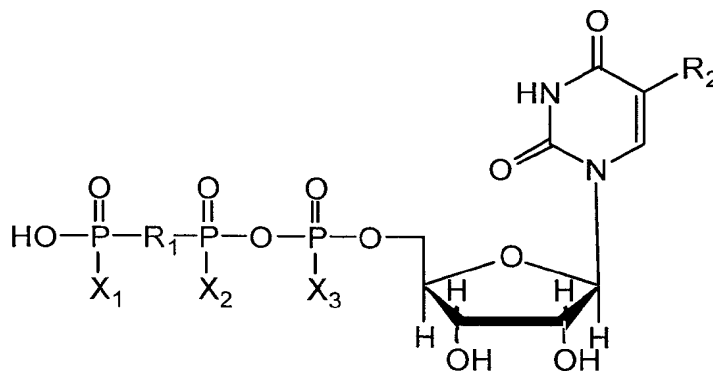
### **DETAILED DESCRIPTION OF THE INVENTION**

Applicants have discovered that nucleotide 5'-triphosphate and dinucleotide polyphosphates are potent agonists for purinergic receptors found in cervical and vaginal epithelia preparations. The methods of the present invention are an improvement upon the current most commonly used treatments of vaginal dryness as compounds of the present invention stimulate a patient's own production and secretion of mucins as well as increasing the levels of mucosal hydration, which serve to maintain the natural protective and lubricant characteristics of vaginal and cervical mucosa. The methods of the present invention may also be used exclusive of, or as an adjunct to, hormone replacement therapy (HRT) or estrogen replacement therapy (ERT).

The present invention provides a method of stimulating cervical and vaginal secretions in a mammal, including a human, in need thereof by administering an amount of a compound of Formulas I, II, III, or IV or a pharmaceutically acceptable ester or salt thereof effective to increase said secretions.

UTP and its analogs are depicted in general Formula I:

Formula I



wherein:

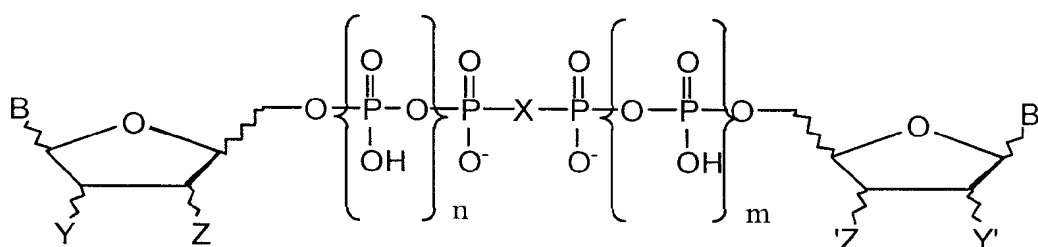
$X_1$ ,  $X_2$  and  $X_3$  are each independently either  $O^-$  or  $S^-$ ; preferably,  $X_2$  and  $X_3$  are  $O^-$ ;

$R_1$  is O, imido, methylene or halomethylene (e.g., dichloromethylene or difluoromethylene); preferably,  $R_1$  is oxygen or difluoromethylene;

5  $R_2$  is H or Br; preferably,  $R_2$  is H; particularly preferred compounds of Formula I are uridine 5'-triphosphate (UTP) and uridine 5'-O-(3-thiotriphosphate) (UTP $\gamma$ S).

A dinucleotide polyphosphate is depicted by the general Formula II:

10

Formula II

wherein:

X is oxygen, methylene, dihaloromethylene, imido;

15

$n = 0, 1, \text{ or } 2$ ;

$m = 0, 1, \text{ or } 2$ ;

$n + m = 0, 1, 2, 3, \text{ or } 4$ ; and

B and B' are each independently a purine residue or a pyrimidine residue linked through the 9- or 1- position, respectively;

20

$Z = OH \text{ or } N_3$ ;

$Z' = OH \text{ or } N_3$ ;

$Y = H \text{ or } OH$ ;

$Y' = H \text{ or } OH$ ;

provided that when Z is  $N_3$ , Y is H or when Z' is  $N_3$ , Y' is H.

25

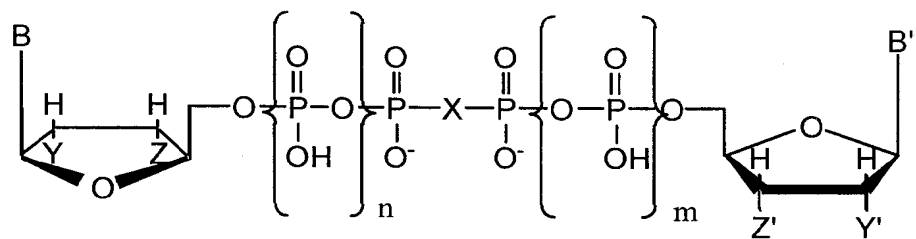
The furanose sugar is preferably in the  $\beta$ -configuration.

The furanose sugar is most preferably in the  $\beta$ -D-configuration.

Preferred compounds of Formula II are the compounds of Formula IIa:



Formula IIa



wherein:

$X=O$ ;

5  $n+m=1$  or  $2$ ;

$Z, Z', Y,$  and  $Y'=OH$ ;

$B$  and  $B'$  are defined in Formulas IIc and IId; or

$X=O$ ;

10  $n+m=3$  or  $4$ ;

$Z, Z', Y,$  and  $Y'=OH$ ;

$B=uracil$ ;

$B'$  is defined in Formulas IIc and IId; or

15  $X=O$ ;

$n+m=1$  or  $2$ ;

$Z, Y,$  and  $Z'=OH$ ;

$Y'=H$ ;

$B=uracil$ ;

20  $B'$  is defined in Formulas IIc and IId; or

$X=O$ ;

$n+m=0, 1,$  or  $2$ ;

$Z$  and  $Y=OH$ ;

25  $Z'=N_3$ ;

$Y'=H$ ;

$B=uracil$ ;

$B'=thymine$ ; or

30  $X=O$ ;

$n+m=0, 1, \text{ or } 2;$

$Z \text{ and } Z'=\text{N}_3;$

$Y \text{ and } Y'=\text{H};$

$B \text{ and } B'=\text{thymine}; \text{ or}$

5

$X=\text{CH}_2, \text{CF}_2, \text{ or } \text{NH};$

$n \text{ and } m=1;$

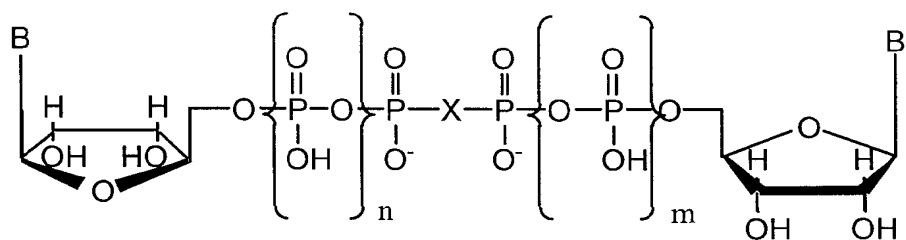
$Z, Z', Y, \text{ and } Y'=\text{OH};$

$B \text{ and } B'$  are defined in Formulas IIc and IId.

10

Another preferred group of the compounds of Formula II are the compounds of Formula IIb or the pharmaceutically acceptable salts thereof:

Formula IIb



15

wherein:

$X$  is oxygen, methylene, difluoromethylene, or imido;

$n = 0 \text{ or } 1;$

$m = 0 \text{ or } 1;$

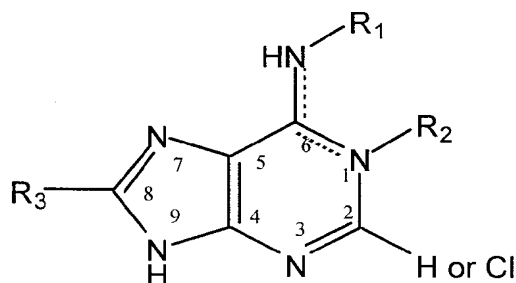
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$n + m = 0, 1, \text{ or } 2; \text{ and}$

$B$  and  $B'$  are each independently a purine residue, as in Formula IIc, or a pyrimidine residue, as in Formula IId, linked through the 9- or 1- position, respectively. In the instance where  $B$  and  $B'$  are uracil, attached at N-1 position to the ribosyl moiety, then the total of  $m + n$  may equal 3 or 4 when  $X$  is oxygen. The ribosyl moieties are in the D- configuration, as shown, but may be L-, or D- and L-. The D- configuration is preferred.

25

30

Formula IIc

5 wherein

$R_1$  is hydrogen,  $C_{1-8}$ alkyl, phenyl, or phenyloxy; wherein at least one hydrogen of said  $C_{1-8}$ alkyl, phenyl, phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy,  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkyl,  $C_{6-10}$ aryl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino,  $C_{1-4}$  alkylamino, di- $C_{1-4}$  alkylamino wherein said alkyl groups are optionally linked to form a heterocycle,  $\omega$ -A( $C_{1-6}$ alkyl)CONH( $C_{1-6}$ alkyl)-, and  $\omega$ -A( $C_{1-6}$ alkyl) NHCO ( $C_{1-6}$ alkyl)-, wherein A is amino, mercapto, hydroxy or carboxyl;

$R_2$  is O or is absent; or

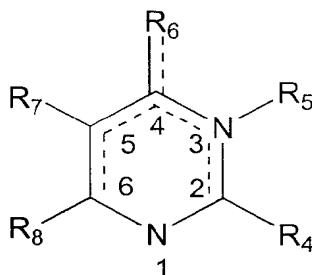
$R_1$  and  $R_2$  taken together form a 5-membered fused imidazole ring optionally substituted on the 4- or 5- positions of the etheno moiety with  $C_{1-4}$ alkyl, phenyl or phenyloxy, wherein at least one hydrogen of said  $C_{1-4}$ alkyl, phenyl, phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy,  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkyl,  $C_{6-10}$ aryl,  $C_{7-12}$ arylalkyl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino,  $C_{1-4}$  alkylamino, and di- $C_{1-4}$  alkylamino wherein said dialkyl groups are optionally linked to form a heterocycle; and

$R_3$  is hydrogen, amino,  $C_{1-8}$ alkyl, phenyl, or phenyloxy; wherein at least one hydrogen of said amino,  $C_{1-8}$  alkyl, phenyl, or phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy,  $C_{1-4}$ alkyl,  $C_{6-10}$ aryl,  $C_{7-12}$ arylalkyl,  $C_{1-4}$ alkoxy,  $C_{7-12}$ arylalkyloxy;  $C_{1-4}$ alkylthio, phenylthio,  $C_{7-12}$ arylalkylthio, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino,  $C_{1-4}$  alkylamino, phenylamino,  $C_{7-12}$ arylalkylamino, di- $C_{1-4}$  alkyl amino wherein said dialkyl groups are optionally linked to form a heterocycle,

$\omega$ -A(C<sub>1-6</sub>alkyl) CONH(C<sub>1-6</sub>alkyl)B-, and  $\omega$ -A(C<sub>1-6</sub>alkyl) NHCO (C<sub>1-6</sub>alkyl)B-, wherein A and B are independently amino, mercapto, hydroxy or carboxyl.

The substituted derivatives of adenine (Formula IIc) include adenine 1-oxide; 1,N6-(4- or 5-substituted etheno) adenine; 6-substituted adenine; or 8-substituted aminoadenine, [6-aminohexyl]carbamoylmethyl-adenine; and  $\omega$ -acylated-amino(hydroxy, thiol and carboxy)alkyl(C<sub>2-10</sub>)-adenine, wherein the acyl group is chosen from among, but not limited to, acetyl, trifluoroacetyl, benzoyl, substituted-benzoyl, etc., or the carboxylic moiety is present as its ester or amide derivative, for example, the ethyl or methyl ester or its methyl, ethyl or benzamido derivative.

#### Formula IIId



wherein:

R<sub>4</sub> is hydrogen, hydroxy, mercapto, amino, cyano, C<sub>7-12</sub>arylalkoxy, C<sub>1-6</sub> alkylthio, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> alkylamino or diC<sub>1-4</sub>alkylamino, wherein the alkyl groups are optionally linked to form a heterocycle;

R<sub>5</sub> is hydrogen, acetyl, benzoyl, C<sub>1-6</sub> alkyl, phenyloxy, C<sub>1-5</sub> alkanoyl, aroyl, or sulphonate;

R<sub>6</sub> is hydroxy, mercapto, C<sub>1-4</sub>alkoxy, C<sub>7-12</sub>arylalkoxy, C<sub>1-6</sub>alkylthio, amino, C<sub>1-5</sub> disubstituted amino, triazolyl, C<sub>1-6</sub>alkylamino, or di-C<sub>1-4</sub>alkylamino wherein said dialkyl groups are optionally linked to form a heterocycle or linked to N<sup>3</sup> to form a substituted ring; or

R<sub>5</sub> and R<sub>6</sub> taken together form a 5-membered fused imidazole ring between positions 3 and 4 of the pyrimidine ring and form a 3,N<sup>4</sup>-ethenocytosine derivative, wherein said etheno moiety is optionally substituted on the 4- or 5- positions with C<sub>1-4</sub> alkyl; phenyl; or phenyloxy; wherein at least one hydrogen of said C<sub>1-4</sub>alkyl; phenyl or phenyloxy is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl, C<sub>6-10</sub>aryl, C<sub>7-12</sub>arylalkyl,

carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C<sub>1-4</sub> alkylamino, and di- C<sub>1-4</sub> alkylamino wherein said dialkyl groups are optionally linked to form a heterocycle;

R<sub>7</sub> is hydrogen, hydroxy, cyano, nitro, or C<sub>2-8</sub>alkenyl; wherein said alkenyl moiety is optionally linked through an oxygen to form a ring, wherein at least one hydrogen of said alkenyl moiety on the carbon adjacent to said oxygen is optionally substituted with C<sub>1-6</sub>alkyl, phenyl, optimally substituted as described below: substituted C<sub>2-8</sub>alkynyl, halogen, substituted C<sub>1-4</sub>alkyl, CF<sub>3</sub>, C<sub>2-3</sub> alkenyl, C<sub>2-3</sub> alkynyl, allylamino, bromovinyl, ethyl propenoate, or propenoic acid; or

R<sub>6</sub> and R<sub>7</sub> together form a 5 or 6-membered saturated or unsaturated ring bonded through N or O at R<sub>6</sub>, such ring optionally contains substituents that themselves contain functionalities; provided that when R<sub>8</sub> is amino or substituted amino, R<sub>7</sub> is hydrogen; and

R<sub>8</sub> is hydrogen, amino or di-C<sub>1-4</sub>alkylamino, C<sub>1-4</sub>alkoxy, C<sub>7-12</sub>arylalkoxy, C<sub>1-4</sub>alkylthio, C<sub>7-12</sub>arylalkylthio, carboxamidomethyl, carboxymethyl, methoxy, methylthio, phenoxy or phenylthio.

In the general structure of Formula IIc above, the dotted lines in the 2- to 6-positions are intended to indicate the presence of single or double bonds in these positions; the relative positions of the double or single bonds being determined by whether the R<sub>4</sub>, R<sub>6</sub>, and R<sub>7</sub> substituents are capable of keto-enol tautomerism.

In the general structures of Formula IIc and IIc above, the acyl groups advantageously comprise alkanoyl or aroyl groups. The alkyl groups advantageously contain 1 to 8 carbon atoms, particularly 1 to 4 carbon atoms optionally substituted by one or more appropriate substituents, as described below. The aryl groups including the aryl moieties of such groups as aryloxy are preferably phenyl groups optionally substituted by one or more appropriate substituents, as described below. The above mentioned alkenyl and alkynyl groups advantageously contain 2 to 8 carbon atoms, particularly 2 to 6 carbon atoms, e.g., ethenyl or ethynyl, optionally substituted by one or more appropriate substituents as described below. Appropriate substituents on the above-mentioned alkyl, alkenyl, alkynyl, and aryl groups are advantageously selected from halogen, hydroxy, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl, C<sub>6-12</sub>aryl, C<sub>6-12</sub>arylalkoxy, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic, amino, and substituted amino wherein the amino is singly or doubly substituted by a C<sub>1-4</sub>alkyl, and when doubly substituted, the alkyl groups optionally being linked to form a heterocycle.

For purposes of further clarifying the foregoing descriptions of Formulae IIc and IId, the descriptions can be simplified to the following:

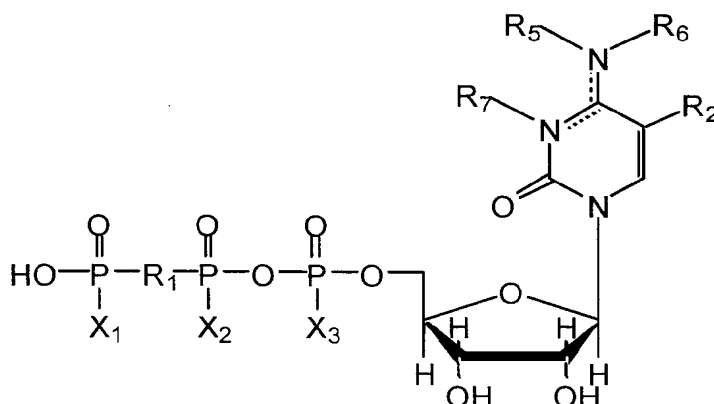
R<sub>2</sub> is O or is absent; or

5 R<sub>1</sub> and R<sub>2</sub> taken together may form optionally substituted 5-membered fused imidazole ring; or

R<sub>1</sub> of the 6-HNR<sub>1</sub> group or R<sub>3</sub> of the 8-HNR<sub>3</sub> group is chosen from the group consisting of:

- (a) hydrogen,
- 10 (b) arylalkyl (C<sub>1-6</sub>) groups with the aryl moiety optionally substituted,
- (c) alkyl,
- (d) [6-(A)-hexyl]carbamoylmethyl, where A is independently chosen from among NH<sub>2</sub>, OH, SH, and CO<sub>2</sub>H,
- 15 (e) ω-amino alkyl (C<sub>2-10</sub>),
- (f) ω-hydroxy alkyl (C<sub>2-10</sub>),
- (g) ω-thiol alkyl (C<sub>2-10</sub>),
- (h) ω-carboxy alkyl (C<sub>2-10</sub>),
- (i) the ω-acylated derivatives of (e), (f), (g), or (h), wherein the
- 20 acyl group is either acetyl, trifluoroacetyl, benzoyl, or substituted-benzoyl alkyl(C<sub>2-10</sub>), and
- (j) ω-carboxy alkyl (C<sub>2-10</sub>) as in (f) above wherein the carboxylic moiety is an ester or an amide.

25 CTP and its analogs are depicted by general Formula III:

Formula III

5 wherein:

$R_1$ ,  $X_1$ ,  $X_2$  and  $X_3$  are defined as in Formula I;

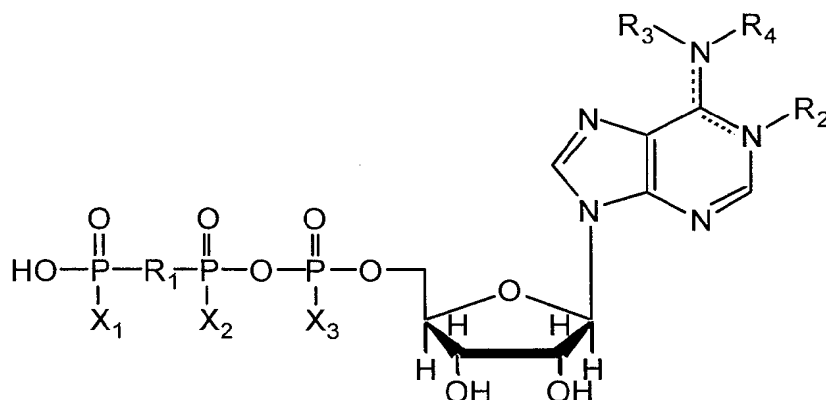
$R_5$  and  $R_6$  are H, while  $R_7$  is absent and there is a double bond between N-3 and C-4 (cytosine), or

10  $R_5$ ,  $R_6$  and  $R_7$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3, $N^4$ -ethenocytosine) optionally substituted at the 4- or 5-position of the etheno ring.

ATP and its analogs are depicted by general Formula IV:

Formula IV

15



wherein:

$R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I;

$R_3$  and  $R_4$  are H, while  $R_2$  is nothing and there is a double bond between N-1 and C-6 (adenine), or

$R_3$  and  $R_4$  are H, while  $R_2$  is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

5         $R_3$ ,  $R_4$ , and  $R_2$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1,N6-ethenoadenine).

For simplicity, Formulas I, II, III, and IV herein illustrate the active compounds in the naturally occurring D-configuration, but the present invention also encompasses compounds in the L-configuration, and mixtures of compounds in the D-  
10 and L-configurations, unless otherwise specified. The naturally occurring D-configuration is preferred.

The compounds of the invention may be present in the form of their pharmaceutically acceptable salts, such as, but not limited to, an alkali metal salt such as sodium or potassium; an alkaline earth metal salt such as manganese, magnesium,  
15 or calcium; or an ammonium or tetraalkyl ammonium salt, i.e.,  $\text{NX}_4^+$  (wherein X is  $\text{C}_{1-4}$ ). Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. The compounds of the invention may also be present in the form of prodrugs, typically comprising esters or amide moieties on the heterocyclic and furanosyl  
20 hydroxyls of the compound.

Another aspect of the present invention is a method of treating a mammal with vaginal dryness arising from, but not limited to, menopause, childbirth, breastfeeding, chemotherapy or radiotherapy, diabetes mellitus, Sjögren's syndrome, Ehlers-Danlos syndrome, systemic sclerosis and other systemic autoimmune diseases, hysterectomy,  
25 urogenital surgery, psychosomatic disorders, anxiety, psychosexual problems, and pharmacological drug-related side effects.

It is also contemplated that the method of the present invention can be used to increase vaginal moisture and lubrication in healthy women for the purpose of facilitating sexual intercourse. It is further contemplated that the method of the  
30 present invention would be particularly useful for a woman who wished to accommodate a sexual partner who is undergoing treatment with Viagra® or other such drugs used for the treatment of erectile dysfunction.

The present invention further provides pharmaceutical compositions comprising a dosage form containing either  $\text{P2Y}_2$  and/or  $\text{P2Y}_4$  purinergic receptor



agonists selected from the group consisting of general Formula I, for example, uridine 5'-triphosphate (UTP) and its analogs; general Formula II, for example,  $P^1, P^4$ -di(uridine-5') tetraphosphate ( $U_2P_4$ ) and its analogs, general Formula III, for example, cytidine 5'-triphosphate (CTP) and its analogs, and general Formula IV, for example, adenosine 5'-triphosphate (ATP) and its analogs.

The compounds disclosed herein may be administered to the cervical and/or vaginal mucosa of a patient by any suitable means, but are preferably administered by a solution, gel, suspension, cream, foam, pessary, or tablet containing the active compound. Alternatively, the active compounds may be administered by continuous release from a vaginal ring (Stumpf, P., *Obstet. Gynecol.* 75:9S (1990)) or an intrauterine device (Andersson, K., *et al.*, *Obstet. Gynecol.* 79:963 (1992)).

The topical solution, gel, jelly, ointment, cream, foam, pessary, or tablet contain the active compound in a physiologically compatible vehicle, as those skilled in the art of gynecological topical delivery system development can select using conventional criteria.

Solutions formulated for administration to the vagina are usually referred to as irrigations. These are sterile solutions, prepared in a manner typical of sterile injections that are intended for and prepared as a single use sterile solution.

Gel (water-soluble bases) and cream (water-removable bases) may be developed dependent on the transport characteristics of the active. For gel formations, gelling agents (e.g., Polyethylene Glycols, gelatin, tragacanth, and Cellulose derivatives) and preservatives (antimicrobial agents) may be used. Stabilizer, antioxidant and buffer may be added as needed. For example, a suitable gel formulation in this invention includes the active compound, glycerin, a cellulose derivative (e.g., hydroxyethyl cellulose), a preservative (e.g., methylparaben), and water. For cream formulations, emulsion bases may be developed. Petrolatum and stearyl alcohol may be used in the oil phase. Petrolatum contributes to the water-holding ability of the formulation, and stearyl alcohol may serve as an emulsifier. The aqueous phase of an emulsion base may contain water-soluble components of the emulsion system, preservative, stabilizer, antioxidant, buffer, and emulsifier. Glycerin, propylene glycol or polyethylene glycol may also be used to minimize the water loss in the finished product.

Ointments are semi-solid preparations that consist of the active ingredient incorporated into a fatty, waxy, or synthetic base.

Examples of suitable creams include, but are not limited to, water-in-oil and oil-in-water emulsions. Water-in-oil creams may be formulated by using a suitable emulsifying agent with properties similar, but not limited, to those of the fatty alcohols such as cetyl alcohol or cetostearyl alcohol and to emulsifying wax. Oil-in-  
5 water creams may be formulated using an emulsifying agent such as cetomacrogol emulsifying wax. Suitable properties include the ability to modify the viscosity of the emulsion and both physical and chemical stability over a wide range of pH. The water soluble or miscible cream base may contain a preservative system and may also be buffered to maintain an acceptable physiological pH.

10 Foam preparations may be formulated to be delivered from a pressurized aerosol canister, via a suitable applicator, using inert propellants. Suitable excipients for the formulation of the foam base include, but are not limited to, propylene glycol, emulsifying wax, cetyl alcohol, and glyceryl stearate. Potential preservatives include methylparaben and propylparaben.

15 Pessaries are solid unit-dose forms suitably shaped for insertion into the vagina and may either be composed of a base that melts at body temperature or which dissolves when in contact with mucous secretions. Examples of suitable bases include, but are not limited to, theobroma oil, synthetic fat bases (e.g. Witepsol), polyethylene glycols (macrogols), and glycerol suppository basis.

20 Vaginal tablets are composed of the active ingredient contained within a solid dosage form base which may include, but not be limited to, excipients such as lactose, microcrystalline cellulose, corn starch, magnesium stearate, silicon dioxide, and hydroxypropyl methylcellulose.

In addition to the topical method of administration described above, there are  
25 various methods of administering the compounds of the present invention systemically. One such means would involve an aerosol suspension of respirable particles comprised of the active compound, which the subject inhales. The active compound would be absorbed into the bloodstream via the lungs and contact the cervical and/or vaginal tissues in a pharmaceutically effective amount. The respirable  
30 particles may be liquid or solid, with a particle size sufficiently small to pass through the mouth and larynx upon inhalation; in general, particles ranging from about 1 to 10 microns, but more preferably 1-5 microns, in size are considered respirable.

Another means of systemically administering the active compounds to the cervical and vaginal tissues of the subject would involve administering a liquid/liquid

suspension in the form of nasal drops of a liquid formulation, or a nasal spray of respirable particles which the subject inhales. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal drops may be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

Other means of systemic administration of the active compound would involve oral administration, in which pharmaceutical compositions containing compounds of Formulas I, II, III, or IV are in the form of tablets, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, syrups, elixirs or transdermal delivery devices. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with nontoxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, or sodium phosphate; granulating and disintegrating agents, for example, corn starch or alginic acid; binding agents, for example, starch, gelatin, or acacia; and lubricating agents, for example magnesium stearate, stearic acid, or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate, or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

Additional means of systemic administration of the active compound to the cervical and vaginal tissues of the subject would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the cervical and vaginal tissues via systemic absorption and circulation.

The above mentioned means of administration may be applied during or after surgical procedures, for example, intra-operative and post-operative installation.

The quantity of the active compound included in the pharmaceutical composition is an amount sufficient to achieve concentrations of the active compound on the cervical and/or vaginal mucosa of the subject of from about  $10^{-7}$  to about  $10^{-1}$  Moles/liter, and more preferably from about  $10^{-6}$  to about  $10^{-1}$  Moles/liter.

Depending on the solubility of the particular formulation of active compound administered, the daily dose to promote cervical and/or vaginal mucin production and/or hydration may be divided among one or several unit dose administrations. The total daily dose for UTP (for example) may range from 1 to 1000 milligrams, depending upon the age and state of the subject, given at a regimen of up to four times per day or on an as needed basis to address acute exacerbations.

Some compounds of Formulas I, II, III, and IV can be made by methods which are well known to those skilled in the art and in accordance with known procedures (Zamecnik, P.C., *et al.*, *Proc. Natl Acad. Sci. USA* 89:2370-2373 (1992); Ng, K., *et al.*, *Nucleic Acids Res.* 15:3572-3580 (1977); Jacobus, K.M., *et al.*, U.S. Patent No. 5,789,391; and Pendergast, W., *et al.*, U.S. Patent No. 5,837,861; and International Patent Application WO98/34942)). Some are commercially available, for example, from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178. The synthetic methods of U.S. Patent 5,789,391 and International Patent Application WO98/34942 are incorporated herein by reference.

## **EXAMPLES**

### **Example 1: In Vitro short circuit ( $I_{sc}$ ) measurements**

The compound UTP is a potent agonist of  $P2Y_2$  and/or  $P2Y_4$  purinergic receptors in cervical and vaginal tissue preparations by evaluation *in vitro* by administering UTP to the tissue culture sufficient to achieve concentrations of UTP on the mucosa of from about  $10^{-7}$  to about  $10^{-1}$  moles/liter. (Rojanasakul, Y., *et al.*, *Pharm. Res.* 9:1029-34 (1992); Bechgaard, E., *et al.*, *Int. J. Pharm.* 106:237-242 (1994); Gipson, I., *et al.*, *Biol. Reprod.* 56:999-1011, (1997)). Specifically, ovariectomized female white albino New Zealand rabbits are sacrificed and vaginal tissue is removed. The tissue is mounted on a supporting ring and clamped in an Ussing chamber.  $I_{sc}$  is measured as flowing from the epithelial side to the serosal side of the tissue. Approximately half of this current corresponds to chloride movement

through the membrane and hence, this is an accurate measure of the corresponding fluid movement.

**Example 2. Cellular localization of P2Y<sub>2</sub> nucleotide receptor gene expression in monkey endocervical and vaginal epithelial tissues by nonisotopic *in situ* hybridization**

**Tissues.** Study tissues were obtained from the Tulane Regional Primate Research Center (Covington, LA). Tissues included in this study were vagina and cervix. Tissues were removed from a 3.25 year old Indian Rhesus Macaque immediately following death and snap frozen in O.C.T. embedding medium. Frozen tissues were shipped overnight on dry ice and stored at -80°C prior to cryosectioning. Tissues were cut in 5 µm sections and mounted on microscope slides for hematoxylin & eosin (H&E) staining, alcian blue/period acid shift (AB/PAS) staining, and *in situ* hybridization (ISH).

**Assessment of Tissue Sections.** H&E-stained tissue sections were prepared to evaluate the quality and orientation of study tissues. Examination of H&E slides indicated that all tissues were suitable for ISH.

**Riboprobe Synthesis.** A PCR product containing nucleotides 253-651 from a human P2Y<sub>2</sub>-R cDNA was prepared. P2Y<sub>2</sub>-R nucleotides 272-627 were reamplified with PCR primers designed to eliminate flanking plasmid sequences and incorporate either an upstream T3 promotor or a downstream T7 promotor. The resulting PCR products were used to synthesize digoxigenin-labeled riboprobes by *in vitro* transcription (IVT). Antisense and sense riboprobes were synthesized using T7 and T3 RNA polymerases, respectively, in the presence of digoxigenin-11-UTP (Boehringer-Mannheim) using a MEGAscript IVT kit (Ambion) according to the manufacturer. Following IVT, template DNA was degraded with DNase-1, and unincorporated digoxigenin was removed by ultrafiltration. Riboprobe integrity was assessed by electrophoresis through a denaturing polyacrylamide gel. Apparent molecular size was estimated by comparison with the electrophoretic mobility of a 100-1000 base pair RNA ladder (Ambion). Probe yield and labeling was evaluated by blot immunochemistry. Riboprobes were dispensed in 5 µL aliquots and stored at -80°C until used for ISH.

***In Situ* hybridization.** Frozen tissues were cut into 5 µm sections, mounted

on SuperFrost Plus slides (Fisher Scientific), and post-fixed for 15 minutes in 4% paraformaldehyde in PBS at pH 7.4. Sections were prehybridized in the absence of probe, then incubated overnight in hybridization buffer containing 400 ng/mL of either antisense or sense probe. Following hybridization, slides were subjected to a series of post-hybridization stringency washes to reduce nonspecific staining. Hybridization was visualized by immunohistochemistry using alkaline phosphatase-conjugated anti-digoxigenin Fab and nitroblue tetrazolium chloride-bromochloroindolyl phosphate (Boehringer-Mannheim) according to the manufacturer. Tissue sections were counter stained with nuclear fast red. Negative controls included cervix and vagina tissues stained with the sense P2Y<sub>2</sub>-R probe.

**Results.** Both endocervical and vaginal epithelial tissues show positive staining using the antisense probe, but negative staining using the sense control probe. The results demonstrate that endocervical epithelial cells (including goblet cells) and the stratified squamous epithelium lining the vaginal canal contain the message for P2Y<sub>2</sub> receptors.

The results support the rationale of the present invention--that activation of P2Y<sub>2</sub> receptors found in the vaginal and cervical epithelium will stimulate vaginal and cervical secretions in mammals.

### **Example 3. Effects of P2Y<sub>2</sub> agonists on vaginal mucosal health in oophorectomized rabbits.**

The purpose of this experiment is to investigate the effect of U<sub>2</sub>P<sub>4</sub> and dCP<sub>4</sub>U on vaginal mucosal health as assessed by clinical and pathologic indicators in a female animal model under conditions of estrogen deprivation and estrogen replacement.

#### **Assessments**

**Daily Assessments.** Mucosal health is assessed by determination of vaginal pH, vaginal lubrication and vaginal smears according to Hubbard *et al.* (*Lab. Anim. Sci.* 47:36-39 (1997)); Bachman *et al.*, (*Clin. Pract. Sexual* 8:12-17 (1992)) at the same time every day one hour post-dosing. Baseline vaginal pH measurements are recorded using a digital pH meter (Sandhill Scientific). Vaginal lubrication is assessed using pre-weighed, especially designed, tampons and vaginal smears by fixing, staining and visual examination by the pathologist.

**Periodic Assessments.** An objective measure of vaginal lubrication are

determined by inserting a pre-weighed tampon and recording the difference in weight over time. A vaginal smear for cytology (vacuolization, basal, parabasal and superficial cells) is performed at the same time on Days 0 (baseline), 4, 9 and 14, 1 hour post-dosing. Baseline vaginal smears, visual examination of external genitalia are performed. Vaginal smears are fixed, stained with the Papanicolaou stain. They are numerically graded and evaluated for vacuolization and percentages of basal, parabasal, and superficial cells by a pathologist.

**Impression cytology.** Animals from each group are then sacrificed using Pentobarbital (8 ml IV). Impression cytology of endocervix to evaluate goblet cell density is done only after sacrifice. The vagina in its entirety (upper and lower segments) as well as the cervix are removed and opened longitudinally). Impression cytology of vaginal and cervical specimens are collected with strips of polyvinylidene difluoride from the vaginal wall and endocervical surface for mucin evaluation by PAS staining. Vaginal specimens are fixed in neutral-buffered 10% formalin, and then embedded in paraffin, cut at 5µm, and stained with hematoxylin and eosin. Blinded subjective grading is performed on vaginal specimens using a vaginal atrophy index (Hubbard, 1997, Bachman, 1992). Data is collected and analyzed by appropriate statistical methods to ascertain significance.

## **Experimental Protocol**

To evaluate the effect of  $P^1$ ,  $P^4$ -di(uridine 5')-tetraphosphate ( $U_2P_4$ ) and  $\{P^1$ -[5'-(2'-(deoxycytidine))- $P^4$ -(5'-(uridine))]-tetraphosphate ( $dCP_4U$ ) on vaginal goblet cell density in oophorectomized animals with and without estrogen replacement therapy, the following experimental protocol will be performed.

**Animal ovariectomy and estrogen replacement therapy.** New Zealand White female rabbits (4 Kg) are divided into 6 groups of six animals per group. (see Table 1). Group 1 remains intact and untreated (no surgery, no placebo, no drug treatment); it is utilized as a reference to obtain the baseline information. The remaining groups of animals (Groups 2-6) are bilaterally ovariectomized under anesthesia, as described (Hansen *et al.*, *Am. J. Obstet. Gynecol.* 175:1272-1280 (1996); Zandberg *et al.*, *Arterioscler. Thromb. Vasc. Biol.* 18:1844-1854 (1998). Two weeks after ovariectomy, Group 2 receives no treatment, Group 3 receives placebo, Group 4 receives  $P^1$ ,  $P^4$ -di(uridine 5')-tetraphosphate ( $U_2P_4$ ) and Group 5 receives

{P<sup>1</sup>-[5'-(2'-(deoxycytidine))-P<sup>4</sup>-(5'-(uridine))}-tetraphosphate (dCP<sub>4</sub>U). Group 6 is implanted with Azat pump delivering estradiol (200 µg/day, for 14 days). Group 6 is further subdivided into 3 subgroups. Group 6A receives placebo; Group 6B receives U<sub>2</sub>P<sub>4</sub> and Group 6C receives dCP<sub>4</sub>U. The estrogen concentration in the blood from each animal is determined by radioimmunoassay; pre-oophorectomy, 14 days post-oophorectomy, and 14 days post-estrogen replacement therapy. Table 1 summarizes the experimental protocol.

**Table 1. Experimental Protocol to study Effects of P2Y<sub>2</sub> Agonists on Vaginal Mucosal Health in Oophorectomized Rabbits**

8 study groups of 6 rabbits per groups = 48 animals minimum.

Group (#)	Operation	Treatment	Daily Assessment	Additional Assessment (Day 0, 4, 9, 14)
Reference (1)	None	None	pH; moisture, secretions, elasticity, mucosal health (subjective)	objective moisture (tampon), vaginal smears for cytology, impression cytology of endocervix (goblet cell density)
Untreated Control (2)	OvX	None	"	"
Placebo Control (3)	OvX	Placebo	"	"
U <sub>2</sub> P <sub>4</sub> Treatment (4)	OvX	U <sub>2</sub> P <sub>4</sub>	"	"
dCP <sub>4</sub> U Treatment (5)	OvX	dCP <sub>4</sub> U	"	"
Estrogen Replaced 6A	OvX	Placebo	"	"
Estrogen Replaced 6B	OvX	U <sub>2</sub> P <sub>4</sub>	"	"
Estrogen Replaced 6C	OvX	dCP <sub>4</sub> U	"	"

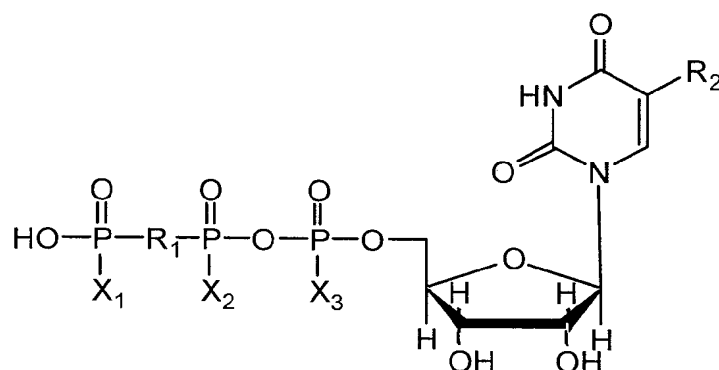
The invention, and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modification may be made therein without departing from the spirit or scope of the



present invention as set forth in the claims.

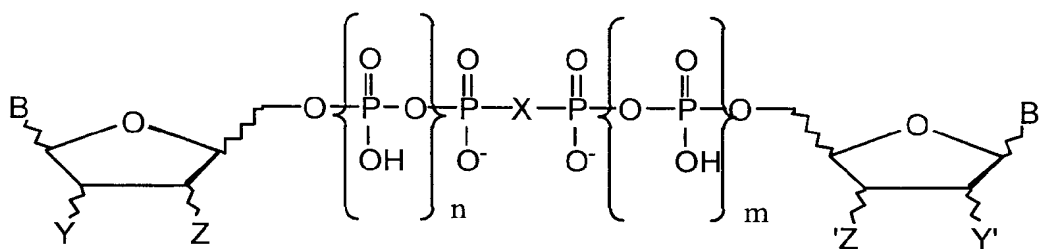
WHAT IS CLAIMED IS:

1. A method of stimulating cervical and vaginal secretions in a mammal in need thereof by administering an effective secretion stimulating amount of a compound of Formulas I, II, III, or IV:

Formula I

wherein:

- $X_1$ ,  $X_2$  and  $X_3$  are each independently either  $O^-$  or  $S^-$ ;  
 $R_1$  is O, imido, methylene or halomethylene;  
 $R_2$  is H or Br; preferably,  $R_2$  is H; or

Formula II

wherein:

- X is oxygen, methylene, difluoromethylene, imido;  
 $n = 0, 1, \text{ or } 2$ ;  
 $m = 0, 1, \text{ or } 2$ ;  
 $n + m = 0, 1, 2, 3, \text{ or } 4$ ; and  
B and B' are each independently a purine residue or a pyrimidine residue linked through the 9- or 1- position, respectively;

$Z = \text{OH or } \text{N}_3$ ;

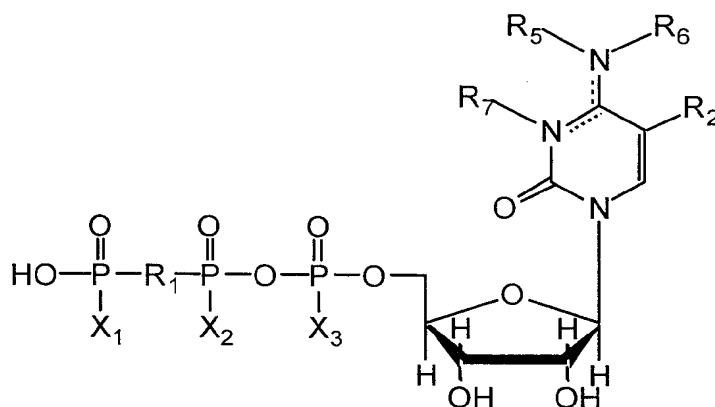
$Z' = \text{OH or } \text{N}_3$ ;

$Y = \text{H or OH}$ ;

$Y' = \text{H or OH}$ ;

5 provided that when  $Z$  is  $\text{N}_3$ ,  $Y$  is H or when  $Z'$  is  $\text{N}_3$ ,  $Y'$  is H; or

Formula III

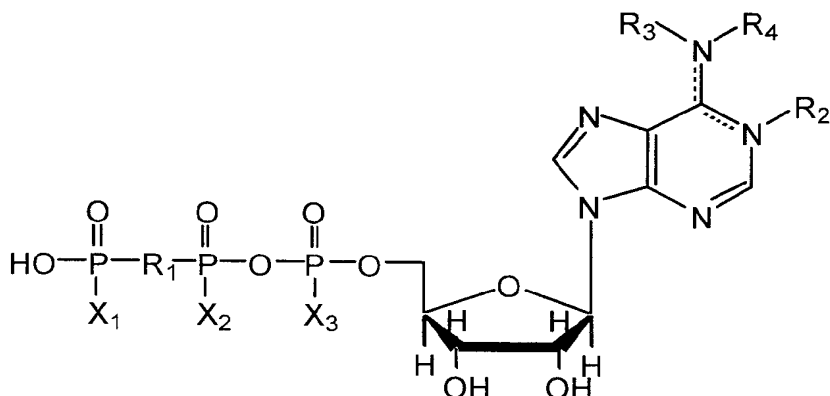


10 wherein:

$R_1$ ,  $X_1$ ,  $X_2$  and  $X_3$  are defined as in Formula I;

$R_5$  and  $R_6$  are H while  $R_7$  is absent and there is a double bond between N-3 and C-4 (cytosine), or

15  $R_5$ ,  $R_6$  and  $R_7$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3, $\text{N}^4$ -ethenocytosine) optionally substituted at the 4- or 5-position of the etheno ring; or

Formula IV

wherein:

5           R<sub>1</sub>, X<sub>1</sub>, X<sub>2</sub>, and X<sub>3</sub> are defined as in Formula I;

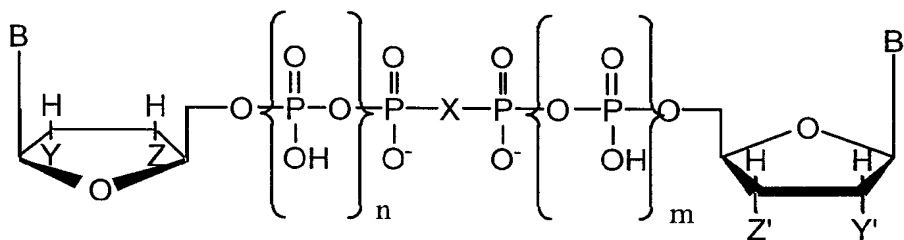
R<sub>3</sub> and R<sub>4</sub> are H while R<sub>2</sub> is absent and there is a double bond between N-1 and C-6, or

R<sub>3</sub> and R<sub>4</sub> are H while R<sub>2</sub> is O and there is a double bond between N-1 and C-6, or

10           R<sub>3</sub>, R<sub>4</sub>, and R<sub>2</sub> taken together are -CH=CH-, forming a ring from N-6 to N-1 with a double bond between N-6 and C-6;

or pharmaceutically acceptable esters or salts thereof.

2.           The method of Claim 1, wherein the compounds of Formula II are  
15   those of Formula IIa:

Formula IIa

wherein:

20           X=O;

n+m=1 or 2;

Z, Z', Y, and Y'=OH;

B and B' are defined in Formulas IIc and IId, or

X=O;

n+m=3 or 4;

5 Z, Z', Y, and Y'=OH;

B=uracil;

B' is defined in Formulas IIc and IId; or

X=O;

10 n+m=1 or 2;

Z, Y, and Z'=OH;

Y'=H;

B=uracil;

B' is defined in Formulas IIc and IId; or

15

X=O;

n+m=0, 1, or 2;

Z and Y=OH;

Z'=N<sub>3</sub>;

20 Y'=H;

B=uracil;

B'=thymine; or

X=O;

25 n+m=0, 1, or 2;

Z and Z'=N<sub>3</sub>;

Y and Y'=H;

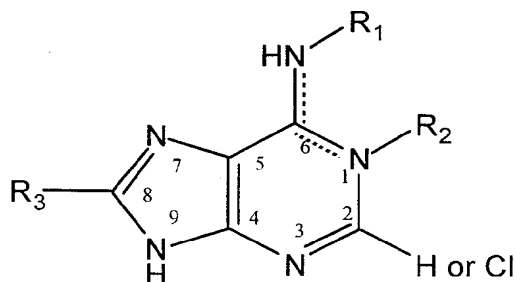
B and B'=thymine; or

30 X=CH<sub>2</sub>, CF<sub>2</sub>, or NH;

n and m=1;

Z, Z', Y, and Y'=OH;

B and B' are defined in Formulas IIc and IId :

Formula IIc

5

R<sub>1</sub> is hydrogen, C<sub>1-8</sub>alkyl, phenyl, or phenyloxy; wherein at least one hydrogen of said C<sub>1-8</sub>alkyl, phenyl, phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl, C<sub>6-10</sub>aryl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C<sub>1-4</sub> alkylamino, di-C<sub>1-4</sub> alkylamino wherein said alkyl groups are optionally linked to form a heterocycle, ω-A(C<sub>1-6</sub>alkyl)CONH(C<sub>1-6</sub>alkyl)-, and ω-A(C<sub>1-6</sub>alkyl) NHCO (C<sub>1-6</sub>alkyl)-, wherein A is amino, mercapto, hydroxy or carboxyl;

R<sub>2</sub> is O or is absent; or

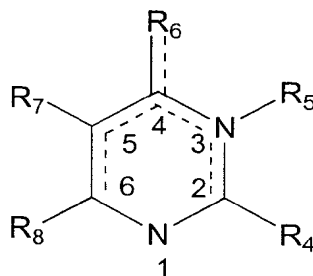
R<sub>1</sub> and R<sub>2</sub> taken together form a 5-membered fused imidazole ring optionally substituted on the 4- or 5- positions of the etheno moiety with C<sub>1-4</sub>alkyl, phenyl or phenyloxy, wherein at least one hydrogen of said C<sub>1-4</sub>alkyl, phenyl, phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkyl, C<sub>6-10</sub>aryl, C<sub>7-12</sub>arylalkyl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C<sub>1-4</sub> alkylamino, and di-C<sub>1-4</sub> alkylamino wherein said dialkyl groups are optionally linked to form a heterocycle; and

R<sub>3</sub> is hydrogen, amino, C<sub>1-8</sub>alkyl, phenyl, or phenyloxy; wherein at least one hydrogen of said amino, C<sub>1-8</sub> alkyl, phenyl, or phenyloxy, is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C<sub>1-4</sub>alkyl, C<sub>6-10</sub>aryl, C<sub>7-12</sub>arylalkyl, C<sub>1-4</sub>alkoxy, C<sub>7-12</sub>arylalkyloxy; C<sub>1-4</sub>alkylthio, phenylthio, C<sub>7-12</sub>arylalkylthio, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C<sub>1-4</sub> alkylamino, phenylamino, C<sub>7-12</sub>arylalkylamino, di-C<sub>1-4</sub> alkyl amino wherein said dialkyl groups are optionally linked to form a heterocycle,

$\omega$ -A(C<sub>1-6</sub>alkyl) CONH(C<sub>1-6</sub>alkyl)B-, and  $\omega$ -A(C<sub>1-6</sub>alkyl) NHCO (C<sub>1-6</sub>alkyl)B-,  
wherein A and B are independently amino, mercapto, hydroxy or carboxyl.

Formula IIc

5



wherein:

R<sub>4</sub> is hydrogen, hydroxy, mercapto, amino, cyano, aralkoxy, C<sub>1-6</sub> alkylthio, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle;

10 R<sub>5</sub> is hydrogen, acyl, C<sub>1-6</sub> alkyl, aroyl, C<sub>1-5</sub> alkanoyl, benzoyl, or sulphonate;

R<sub>6</sub> is hydroxy, mercapto, alkoxy, aralkoxy, C<sub>1-6</sub>-alkylthio, C<sub>1-5</sub> disubstituted amino, triazolyl, alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle or linked to N<sup>3</sup> to form an optionally substituted ring; or

15 R<sub>5</sub> - R<sub>6</sub> together forms a 5 or 6-membered saturated or unsaturated ring bonded through N or O at R<sub>6</sub>, wherein said ring is optionally substituted;

R<sub>7</sub> is selected from the group consisting of:

- (a) hydrogen,
- (b) hydroxy,
- 20 (c) cyano,
- (d) nitro,
- (e) alkenyl, wherein the alkenyl moiety is optionally linked through oxygen to form a ring optionally substituted with alkyl or aryl groups on the carbon adjacent to the oxygen,
- 25 (f) substituted alkynyl
- (g) halogen,
- (h) alkyl,
- (i) substituted alkyl,

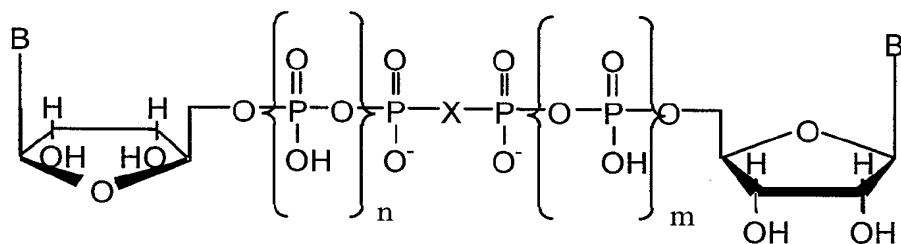
- (j)  $\text{CF}_3$ ,
- (k)  $\text{C}_{2-6}$  alkyl,
- (l)  $\text{C}_{2-3}$  alkenyl,
- (m) substituted ethenyl,
- (n)  $\text{C}_{2-3}$  alkynyl and
- (o) substituted alkynyl;

$\text{R}_8$  is selected from the group consisting of:

- (a) hydrogen,
- (b) alkoxy,
- (c) arylalkoxy,
- (d) alkylthio,
- (e) arylalkylthio,
- (f) carboxamidomethyl,
- (g) carboxymethyl,
- (h) methoxy,
- (i) methylthio,
- (j) phenoxy,
- (k) phenylthio,
- (l) amino,
- (m) alkylamine, and
- (n) dialkylamino.

3. The method of Claim 1, wherein the compounds of Formula II are those of Formula IIb:

Formula IIb



wherein:

X is oxygen, methylene, difluoromethylene, or imido;



$n = 0$  or  $1$ ;

$m = 0$  or  $1$ ;

$n + m = 0, 1, \text{ or } 2$ ; and

B and B' are each independently a purine residue, as in Formula IIc as  
5 described in claim 2, or a pyrimidine residue, as in Formula IId as described in claim  
2, linked through the 9- or 1- position, respectively; provided that when B and B' are  
uracil, attached at N-1 position to the ribosyl moiety, then the total of  $m + n$  equals 3  
or 4 when X is oxygen.

10 4. The method of Claim 1, wherein  $R_2$  of Formula I is H.

5. The method of Claim 1, wherein the furanose sugar of Formula II is in  
the  $\beta$ -D-configuration.

15 6. A method of treating a mammal with vaginal dryness by administering  
an effective vaginal dryness treatment amount of a compound of Formulas I, II, III, or  
IV as described in Claims 1-5.

20 7. A pharmaceutical composition comprising a compound of Formulas I,  
II, III, or IV as described in claims 1-5 together with a pharmaceutically acceptable  
carrier therefor in the form of a liquid or gel suspension.

25 8. The method of Claim 6, wherein the amount of compound of Formulas  
I, II, III or IV administered to the mammal is sufficient to achieve a concentration on  
the cervical and/or vaginal mucosa of from about  $10^{-7}$  moles/liter to about  $10^{-1}$   
moles/liter.

30 9. The method of Claim 6, wherein the amount of compound of Formulas  
I, II, III, or IV administered to the mammal is sufficient to achieve a daily dose of  
between 1 to 1000 milligrams.

10. A method of treating a mammal with vulvar pain by administering an  
effective vulvar pain treatment amount of a compound of Formulas I, II, III, or IV as  
described in Claims 1-5.